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SEASONAL FLUCTUATIONS IN ACTIVITY OF HUMAN BONE MARROW STROMAL PRECURSOR CELLS

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Circadian and circennial changes in physiological processes in man and animals have recently been described, and in particular, seasonal changes have been shown to take place in the peripheral blood leukocyte count, activity of adrenocortical and sex hormones, and levels of certain vitamins [2, 3]. Exacerbations of rheumatic fever and rheumatoid arthritis in the spring and fall were first observed long ago. However, there are no data on seasonal changes in activity of cells possessing stem qualities, which include stromal precursor cells (CFU-F) of the bone marrow, which play an important role in bone regeneration and in the formation of the hematopoietic microenvironment [5, 6].

The aim of this investigation was to study CFU-F activity of human bone marrow in the course of the year, based on the results of cloning in vitro.

EXPERIMENTAL METHOD

The cloning efficiency of bone marrow CFU-F was investigated in 250 orthopedic patients over a period of observation lasting 7 years: from February, 1979, through July, 1985. The results of cloning of CFU-F from the bone marrow of the sternum, iliac crest, tibial tuberosity, and other parts of the skeleton in patients with lesions of the bones and joints (osteomyelitis, aseptic necrosis, pseudarthrosis, arthritis deformans, congenital dislocation of the hip, and so on), but with no evidence of degenerative, dystrophic, or inflammatory changes at the site from which the material was obtained, were subjected to analysis. Under these circumstances the results could be pooled and analyzed together.

Cloning of CFU-F was carried out in 73 cases without a feeder and in 177 cases with a rabbit feeder by the method described in [1]. Culture was carried out in glass flasks (area of bottom 69 and 85 cm^2) in medium 199 with 15-20% human group AB (IV) serum for 12-14 days. The growing colonies were fixed with ethanol and stained by the Romanovsky–Giemsa method. Cloning efficiency was determined by the number of growing colonies per 10^5 transplanted human nucleated bone marrow cells. Concentrations of fibroblast-like cells containing at least 50 cells were taken to be colonies [6]. The results were subjected to statistical analysis by nonparametric tests [4].

EXPERIMENTAL RESULTS

The results of analysis of cloning of human CFU-F month by month for a year are given in Table 1.

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TABLE 1. Seasonal Fluctuations in Bone Marrow CFU-F Activity (1979-1985)

Cloning efficiency of CFU-F per 10^5 nucleated cells	January	February	March	April	May	June	July	August	September	October	November	December
0	2	2	8	12	4	2	0	3	4	13	0	1
Under 20	19	6	9	6	13	7	7	18	16	5	3	1
20—40	3	1	1	1	5	5	1	2	3	0	4	4
40—60	4	0	0	0	2	1	4	2	1	1	2	5
60—80	2	2	3	1	2	3	0	0	2	1	0	1
80—100	0	1	0	0	0	0	0	0	0	2	0	2
Over 100	2	0	0	0	3	1	0	0	1	4	3	2
Total . . .	32	12	21	20	29	19	11	25	27	26	12	16/250

Legend. Number of observations given in Table.

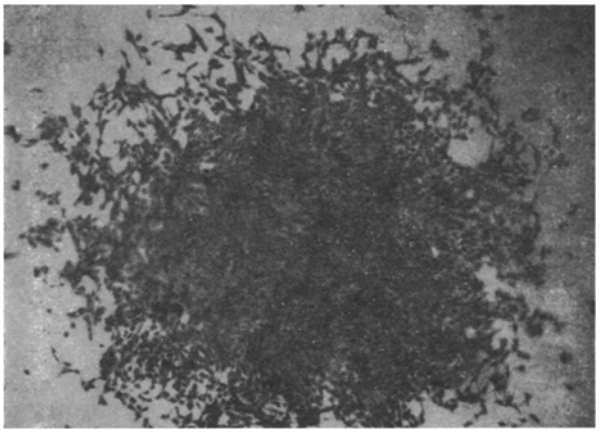

Fig. 1. Colony of stromal fibroblasts of human bone marrow. 100×.

The number of negative results of cloning, which was altogether 20.4%, was significantly higher in the case of cloning without a feeder (32.9%) than with a feeder (15.2%), and it was unevenly distributed in the course of the year. For instance, in March, April, and October the number of zero results of CFU-F cloning was 38, 60, and 50% respectively, whereas in the other months of the year (July and November) colonies of fibroblast-like cells grew in all the patients investigated. Four zero results were obtained in January and February during cloning without a feeder, whereas during cloning with a feeder, in 44 cases there was none in which colonies of fibroblast-like cells did not grow.

Representation of the ratio of the number of zero results to the total number of investigations month by month in graphic forms shows two peaks of lowering of CFU-F activity in the bone marrow: in April and October ($p < 0.01$).

At the $p = 0.01$ level the confidence interval of the fraction of zero results of CFU-F cloning was 32.4-84.6% in April and 25.3-74.7% in October. The minimal calculated frequencies of zero results during these months exceeded the percentage of negative results in the remaining months of the year (6.25-16.7%) with the exception of March (38.1%). This indicates significance of the differences observed, and this is confirmed also by analysis of the data by Fisher's fourfold distribution method.

Analysis of the values of cloning efficiency of bone marrow CFU-F for the 1979-1985 period during cloning with rabbit feeder (177 cases) showed that not only is a high percentage of zero results observed in March and April, but cloning efficiency of CFU-F as a whole was shifted toward lower values. For instance, in April, of seven investigations the cloning efficiency of CFU-F in four cases was under 5 per 10^5 nucleated cells. In March and April, in none of the 19 cases was the cloning efficiency of CFU-F above 70 per 10^5 nucleated cells and it varied between 0.25 and 68 per 10^5 nucleated cells. A rather different picture was observed in October. Not only did the number of zero results in that month reach 50%, but

no low values of cloning efficiency (below 40 per 10^5 nucleated cells) were recorded in the case of culture with feeder. High values of cloning efficiency of CFU-F (over 100 per 10^5 nucleated cells) were obtained in October in four of eight cases; a high percentage of large stratified colonies also was observed (Fig. 1) in that month.

The cyclic character of CFU-F activity in the bone marrow thus revealed affects the course of the postoperative period in these patients. For instance, of 50 patients with osteomyelitis investigated, 14 had complications in the postoperative period or had an unsuccessful operation. In 11 of them cloning efficiency of CFU-F was zero. Nine patients underwent their operation in March-April and October ($p < 0.01$), five in August, February, and November.

The cyclic changes in CFU-F activity in the bone marrow in the course of the year thus reflects complex physiological processes in the body, which are seasonal in character, and whose causes and mechanisms require further intensive investigation. These changes must be taken into account when the physiology and pathology of human bone and hematopoietic tissues are studied and also in clinical practice.

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